A CLEAN SET OF CLAIMS

1. An in vitro immunoassay method for diagnosing human colonic type gastric intestinal metaplasia which comprises the steps of:

(a) contacting a gastric tissue sample of a subject suspected of having human colonic type gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen, wherein the gastric tissue is not a gastric cardia; and

(b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human colonic type gastric intestinal metaplasia.

2. The method according to claim 1, wherein the human gastric intestinal metaplasia antigen is colon epithelial specific protein.

3. The method according to claim 1, wherein the antibody or fragment is directly attached to a detectable label.

4. The method according to claim 1, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectronmicroscopy, or ELISA.

5. The method according to claim 4, wherein the immunoassay method is immunoperoxidase staining.

6. The method according to claim 5, wherein the immunoperoxidase staining comprises:

(a) deparaffinizing the gastric tissue by heating;

(b) immersing the deparaffinized tissue in xylene;

(c) rehydrating the tissue in decreasing concentrations of alcohol;

(d) washing the rehydrated tissue in neutral PBS;

(e) reducing the aldehydes of the washed tissue of step (d);

(f) reacting the tissue with normal goat serum, the monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;

(g) treating the reacted tissue with diaminobenzidine;

(h) washing the diaminobenzidine-treated tissue;

(i) staining the washed tissue of step (h) with hematoxylin, eosin or both; and

(o) examining the stained tissue under a microscope to detect the presence of immunoreactivity.

7. The method according to claim 6, which further comprises the step of trypsinizing the gastric tissue after reducing the aldehydes in the tissue but before

reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

8. The method according to claim 6, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

9. The method according to claim 1, further comprising the step of performing a negative control assay on a negative control sample to detect cells in the gastric tissue sample of the subject suspected of having human colonic type gastric intestinal metaplasia and comparing results of the gastric tissue sample with the results of the negative control sample, wherein the presence of human colonic type gastric intestinal metaplasia cells in the gastric tissue sample over the absence of human colonic type gastric intestinal metaplasia cells in the negative control sample indicates a positive diagnosis of human colonic type gastric intestinal metaplasia.

10. The method according to claim 1, further comprising the step of performing a positive control assay on a positive control sample to detect human cells of colonic type gastric intestinal metaplasia present in the positive control sample.

11. An in vitro immunoassay method for screening for human colonic type gastric intestinal metaplasia, wherein reactivity with DAS-1 is indicative of a predisposition for gastric carcinoma, which comprises the steps of:

(a) contacting a gastric tissue sample of a subject suspected of having human colonic type gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced

by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen, wherein the gastric tissue is not a gastric cardia; and

(b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human colonic type gastric intestinal metaplasia.

12. The method according to claim 11, wherein the human gastric intestinal metaplasia antigen is colon epithelial specific protein.

13. The method according to claim 11, wherein the antibody or fragment is directly attached to a detectable label.

14. The method according to claim 11, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectromicroscopy, or ELISA.

15. The method according to claim 11, wherein the immunoassay method is immunoperoxidase staining.

16. The method according to claim 15, wherein the immunoperoxidase staining comprises:

(a) deparaffinizing the gastric tissue by heating;

(b) immersing the deparaffinized tissue in xylene;

(c) rehydrating the tissue in decreasing concentrations of alcohol;

(d) washing the rehydrated tissue in neutral PBS;

(e) reducing the aldehydes of the washed tissue of step (d);

(f) reacting the tissue with normal goat serum, the monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;

(g) treating the reacted tissue with diaminobenzidine;

(h) washing the diaminobenzidine-treated tissue;

(i) staining the washed tissue of step (h) with hematoxylin, eosin or both; and

examining the stained tissue under a microscope to detect the presence of immunoreactivity.

NE

17. The method according to claim 16, which further comprises the step of trypsinizing the gastric tissue after reducing the aldehydes in the tissue but before reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

18. The method according to claim 16, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

19. The method according to claim 16, further comprising the step of performing a negative control assay on a negative control sample to detect cells in the gastric tissue sample of the subject suspected of having human colonic type gastric intestinal metaplasia and comparing results of the gastric tissue sample with the results of the negative control sample, wherein the presence of human colonic type gastric intestinal metaplasia cells in the gastric tissue sample over the absence of human colonic type gastric intestinal metaplasia cells in the negative control sample indicates a positive diagnosis of human colonic type gastric intestinal metaplasia..

20. The method according to claim 16, further comprising the step of performing a positive control assay on a positive control sample to detect human cells of colonic type gastric intestinal metaplasia present in the positive control sample.

A Mark-Up Version of Claims

1. [currently amended] An in vitro immunoassay method for diagnosing human colonic type gastric intestinal metaplasia which comprises the steps of:

(a) contacting a gastric tissue sample of a subject suspected of having human colonic type gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen, wherein the gastric tissue is not a gastric cardia; and

(b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human colonic type gastric intestinal metaplasia.

2. [previously amended] The method according to claim 1, wherein the human gastric intestinal metaplasia antigen is colon epithelial specific protein.

3. [previously amended] The method according to claim 1, wherein the antibody or fragment is directly attached to a detectable label.

4. [original] The method according to claim 1, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectronmicroscopy, or ELISA.

5.[original] The method according to claim 4, wherein the immunoassay method is immunoperoxidase staining.

6. [previously amended] The method according to claim 5, wherein the immunoperoxidase staining comprises:

(a) deparaffinizing the gastric tissue by heating;

(b) immersing the deparaffinized tissue in xylene;

(c) rehydrating the tissue in decreasing concentrations of alcohol;

(d) washing the rehydrated tissue in neutral PBS;

(e) reducing the aldehydes of the washed tissue of step (d);

(f) reacting the tissue with normal goat serum, the monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;

(g) treating the reacted tissue with diaminobenzidine;

(h) washing the diaminobenzidine-treated tissue;

(i) staining the washed tissue of step (h) with hematoxylin, eosin or both; and

(o) examining the stained tissue under a microscope to detect the presence of immunoreactivity.

7. [previously amended] The method according to claim 6, which further comprises the step of trypsinizing the gastric tissue after reducing the aldehydes in the

tissue but before reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

8.[original] The method according to claim 6, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

9. [currently amended] The method according to claim 1, further comprising the step of performing a negative control assay on a negative control sample to detect cells ~~of human colonic type gastric intestinal metaplasia present in the negative control sample~~ in the gastric tissue sample of the subject suspected of having human colonic type gastric intestinal metaplasia and comparing results of the ~~assay in (b)~~ gastric tissue sample with the results of the negative control ~~assay~~ sample, wherein the presence of human colonic type gastric intestinal metaplasia cells in the ~~assay in (b) above~~ gastric tissue sample over the ~~presence~~ absence of human colonic type gastric intestinal metaplasia cells in the negative control ~~assay~~ sample indicates a positive diagnosis of human colonic type gastric intestinal metaplasia.

10. [previously amended] The method according to claim 1, further comprising the step of performing a positive control assay on a positive control sample to detect human cells of colonic type gastric intestinal metaplasia present in the positive control sample.

11. [currently amended] An in vitro immunoassay method for screening for human colonic type gastric intestinal metaplasia, wherein reactivity with DAS-1 is indicative of a predisposition for gastric carcinoma, which comprises the steps of:

(a) contacting a gastric tissue sample of a subject suspected of having human colonic type gastric intestinal metaplasia cells with the monoclonal antibody DAS-1, or a fragment thereof, which monoclonal antibody is produced by the hybridoma deposited under ATCC accession number HB 9397 and which reacts with human gastric intestinal metaplasia antigen, wherein the gastric tissue is not a gastric cardia; and

(b) detecting immunoreactivity between the gastric tissue and the monoclonal antibody, such immunoreactivity indicating a positive diagnosis of human colonic type gastric intestinal metaplasia.

12. [previously amended] The method according to claim 11, wherein the human gastric intestinal metaplasia antigen is colon epithelial specific protein.

13. [previously amended] The method according to claim 11, wherein the antibody or fragment is directly attached to a detectable label.

14.[original] The method according to claim 11, wherein detecting immunoreactivity is performed by immunoperoxidase staining, immunofluorescence, immunoelectromicroscopy, or ELISA.

15.[original] The method according to claim 11, wherein the immunoassay method is immunoperoxidase staining.

16. [previously amended] The method according to claim 15, wherein the immunoperoxidase staining comprises:

(a) deparaffinizing the gastric tissue by heating;

(b) immersing the deparaffinized tissue in xylene;

(c) rehydrating the tissue in decreasing concentrations of alcohol;

(d) washing the rehydrated tissue in neutral PBS;

(e) reducing the aldehydes of the washed tissue of step (d);

(f) reacting the tissue with normal goat serum, the monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex;

(g) treating the reacted tissue with diaminobenzidine;

(h) washing the diaminobenzidine-treated tissue;

(i) staining the washed tissue of step (h) with hematoxylin, eosin or both; and

examining the stained tissue under a microscope to detect the presence of immunoreactivity.

17. [previously amended] The method according to claim 16, which further comprises the step of trypsinizing the gastric tissue after reducing the aldehydes in the tissue but before reacting the tissue with the goat serum, monoclonal antibody, biotinylated goat anti-mouse antibody and avidin-biotin-peroxidase complex.

18.[original] The method according to claim 16, wherein the decreasing concentrations of alcohol used for rehydration are 100%, 95%, 70%, and 50% alcohol.

19. [currently amended] The method according to claim 16, further comprising the step of performing a negative control assay on a negative control sample to detect cells ~~of human colonic type gastric intestinal metaplasia present in the negative control sample~~ in the gastric tissue sample of the subject suspected of having human colonic type gastric intestinal metaplasia] and comparing results of the ~~assay in (b)~~ gastric tissue sample with the results of the negative control ~~assay~~ sample, wherein the presence of human colonic type gastric intestinal metaplasia cells in the ~~assay in (b) above~~ gastric tissue sample over the ~~presence~~ absence of human colonic type gastric intestinal metaplasia cells in the negative control ~~assay~~ sample indicates a positive diagnosis of human colonic type gastric intestinal metaplasia.

20. [previously amended] The method according to claim 16, further comprising the step of performing a positive control assay on a positive control sample to detect human cells of colonic type gastric intestinal metaplasia present in the positive control sample.